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The Acrosome Reaction and Fertilization in the Bivalve, *Laternula limicola*, in Reference to Sperm Penetration from the Posterior Region of the Mid-piece

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ABSTRACT—The processes of acrosome reaction and sperm penetration in the bivalve *Laternula limicola* were observed using light and electron microscopy. The spermatozoon of *Laternula limicola* is unique with respect to the position of the acrosome. The acrosome of the mature spermatozoon is situated posterior to the mitochondria in the sperm mid-piece. The acrosome consists of a bipartite structure, the acrosomal vesicle and the subacrosomal region. In the first stage of the acrosome reaction, a pit forms at the posterior tip of the acrosomal vesicle. In the second stage, the outer membrane of the acrosomal vesicle and the sperm plasma membranes fuse at the lateral part of the acrosome and open. In the third stage, the plasma membrane continually degrades and the inner acrosomal membrane is exposed. In the fourth stage, the exposed inner acrosomal membrane contacts the apex of an egg microvillus. At the site of contact, a fertilization cone is rapidly formed. Sperm incorporation in this species first begins at the posterior lateral region of the sperm mid-piece, not at the anterior region of the sperm head in the manner of many other species, because the acrosome lies at the most posterior end of the mid-piece. Sperm incorporation from the mid-piece has not been previously described. The present report on normal fertilization is the first description of the morphological changes in the acrosome of *Laternula limicola* spermatozoa and sperm incorporation beginning at the posterior region of the sperm mid-piece.

INTRODUCTION

The mature spermatozoon in most animals is fundamentally composed of three distinct parts, the head, mid-piece and tail. Reports on the morphology of spermatozoa have so far described the nucleus occupying the main portion of sperm head and the acrosome as situated at the apex of the head [38], except in species such as the Ostheichthyes [3, 43, 48, 53], Thaliacea [17], Crustacea [42], Kinorhyncha [24, 39], Nematodea [5, 22, 34], Cestoidea [32] and Anthozoan [13], in which lack an acrosome. The actual structure of the intact acrosome varies morphologically from one species to another.

Since Dan [9] first stated that the acrosome reaction plays an important role in fertilization, many subsequent experiments have confirmed that the acrosome reaction is essential for the success of sperm entry in species in which the spermatozoa possess an acrosome. During the normal course of fertilization in many species, including those lacking acrosome, spermatozoa initially contact the egg or the oocyte in the region of the sperm head and enter the ooplasm starting at the head [1, 15, 21, 31, 35, 44, 45, 49, 50, 56].

The fine structure of spermatozoa of the bivalve, *Laternula limicola,* was first observed by Kubo in 1977 [25]. Kubo reported that mature spermatozoa of this species do not have a "typical acrosome" situated at the anterior area of the head, but have an acrosome-like structure that is uniquely located at the sperm mid-piece and assigned it the new name of "temporary acrosome". However, the role of the posterior temporary acrosome in fertilization was not defined in this report.

For this reason, the authors studied the process of fertilization in the bivalve, *Laternula limicola,* with particular attention to whether or not any morphological changes occur in the posterior temporary acrosoma structure and to what region of the sperm first touches and fuses with the egg plasma membrane.

MATERIALS AND METHODS

Specimens of the bivalve *Laternula limicola* (REEVE) were collected from a tideland in the vicinity of the Imakiri River mouth in Tokushima prefecture Japan during August and September from 1986 to 1989. The shells of this species are frail, light gray in color, and elliptical in shape, measuring about 35 mm along the major axis and 15 mm along the minor axis. Since this species is a hermaphrodite, the ovotestis concurrently matures during the breeding season.

After the shells were removed, the bodies of mature adult specimens were placed in a Petri dish filled with 80% sea water containing 0.1 M acetylcholine. This treatment induced spawning of mature spermatozoa and prophase I oocytes from the parallel openings of the oviduct and spermiduct. The spermatozoa and the oocytes were separately collected by a pipette before the spermatozoa could disperse. The spermatozoa were quickly placed in a Petri dish separate from the oocytes. The oocytes were inseminated in 80% sea water.

To examine the interaction between the spermatozoon and the oocyte, the fertilization process in specimens was observed using a light microscope within 8 min after insemination. To induce the acrosome reaction, the spermatozoa were exposed to Ionophore

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A23187. Ionophore A23187 was dissolved in dimethyl sulfoxide and diluted as a 40 μg/ml solution in sea water. An equal volume of Ionophore solution was added to the sperm suspension. These specimens were used for electron microscopic observations.

The specimens were fixed at room temperature for 2 hr in 2.5% glutaraldehyde buffered to pH 7.2 with 0.1 M cacodylate. They were then post-fixed for 1 hr in 1% osmium tetroxide. After they were dehydrated through a graded acetone series, the specimens were embedded in an Epoxy 812 resin mixture. Ultra-thin sections were stained with uranyl acetate and lead citrate and observed with a Hitachi H-600 transmission electron microscope operated at 100 KV. For scanning electron microscopic observations, fixed specimens were dried with a critical point drying apparatus, coated with gold by ion sputtering and observed with a Hitachi 450 DX scanning electron microscope operated at 15 KV. Light microscopic observations of living materials were conducted with Nomarski differential interference-contrast optics.

OBSERVATIONS

1 Spermatozoon and oocyte

The mature spermatozoon of *L. limicola* is composed of three parts; a tapering head about 5 μm in length and 1 μm in width, a large mid-piece about the same length as the head and a long flagellated tail about 60 μm in length (Figs. 1a, b). The mid-piece contains five mitochondria accompanied by the acrosome, which lies posterior to the mitochondria and a pair of centrioles, the proximal and distal centrioes (Fig. 2a). This spermatozoon has a clearly discernible dorsoventral asymmetry due to the dorsal location of the distal centriole, from which the axonemal complex arises, and the ventral location of the proximal centriole. The distal centriole is not surrounded by the five mitochondria. The sperm flagellum is separated dorsally from the mid-piece and extends parallel to the mid-piece. The basal body which is elongated from the distal centriole at the base of the axonemal filament is about 4 μm in length.

The acrosome is situated posterior to the five mitochondria and is comprised of a bipartite structure, the acrosomal vesicle and the subacrosomal region. The acrosomal vesicle is an elongated bell-shaped structure enclosed by a limiting membrane in longitudinal section. The subacrosomal region is contained within a 3 μm deep invagination of the acrosomal vesicle (Fig. 2b). The homogeneous materials enclosed within the acrosomal vesicle are more electron dense than the materials of the subacrosomal region.

During the breeding season, *L. limicola* spawn primary

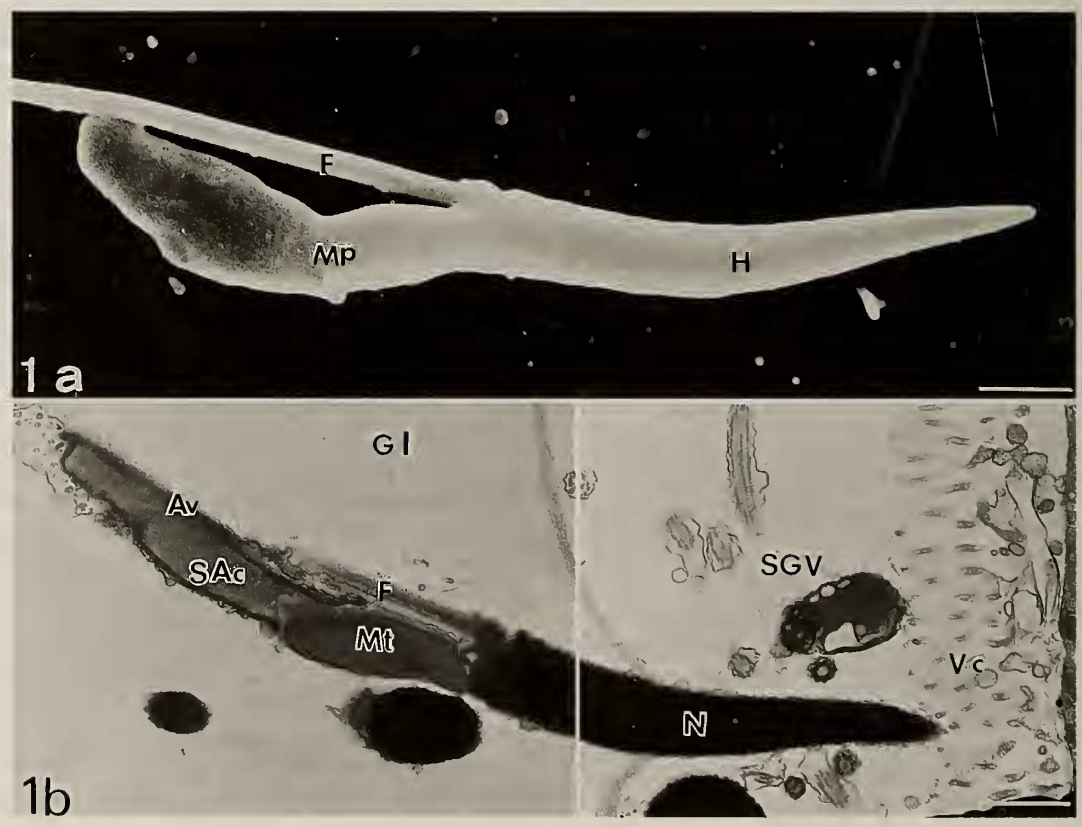


FIG. 1. Electron micrographs of whole views of mature spermatozoa of *Laternula limicola*.
a: Scanning electron micrograph (SEM) of the spermatozoon. The flagellum (F) is separated dorsally from the mid-piece (Mp). Bar: 1 μm. b: Transmission electron micrograph (TEM) of a spermatozoon travelling from the granular layer (Gl) of the egg investments to the SGV. It has a large acrosome posterior to the mitochondrial mid-piece and ventral to the flagellum. The acrosome is comprised of the acrosomic vesicle (Av) and the subacrosomal region (SAc). The acrosome reaction has not yet begun. Bar: 1 μm.
H: Head, Mt: Sperm mitochondria, N: Nucleus, SGV: Space between granular layer and vitelline coat, Vc: Vitelline coat

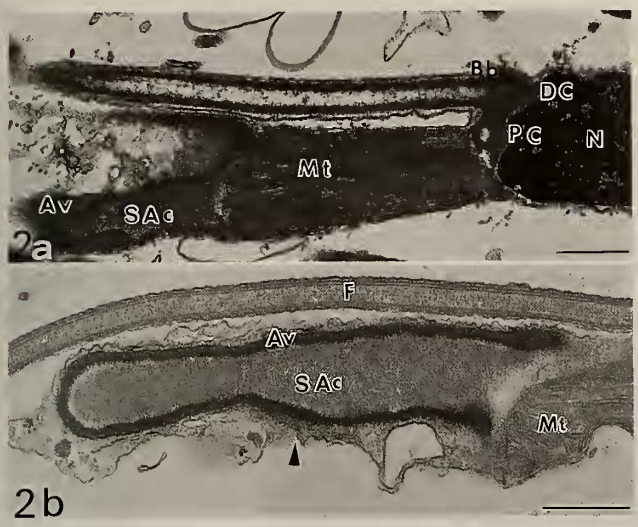


FIG. 2. Micrographs (TEM) of longitudinal sections through the acrosomal structure posterior to the mitochondrial mid-piece.
a: A pair of centrioles with the distal centriole (DC) dorsal and the proximal centriole (PC) ventral. The basal body (Bb) elongated from the distal centriole. Bar: 0.5 μm.
b: An intact acrosomal vesicle (Av) forming a slender bell-shaped sheath around the subacrosomal region (SAc). The acrosomal vesicle is more electron dense than the subacrosomal region. An arrowhead indicates the ventral lateral part of the acrosome. Bar: 0.5 μm.
F: Flagellum, Mt: Sperm mitochondria, N: Nucleus

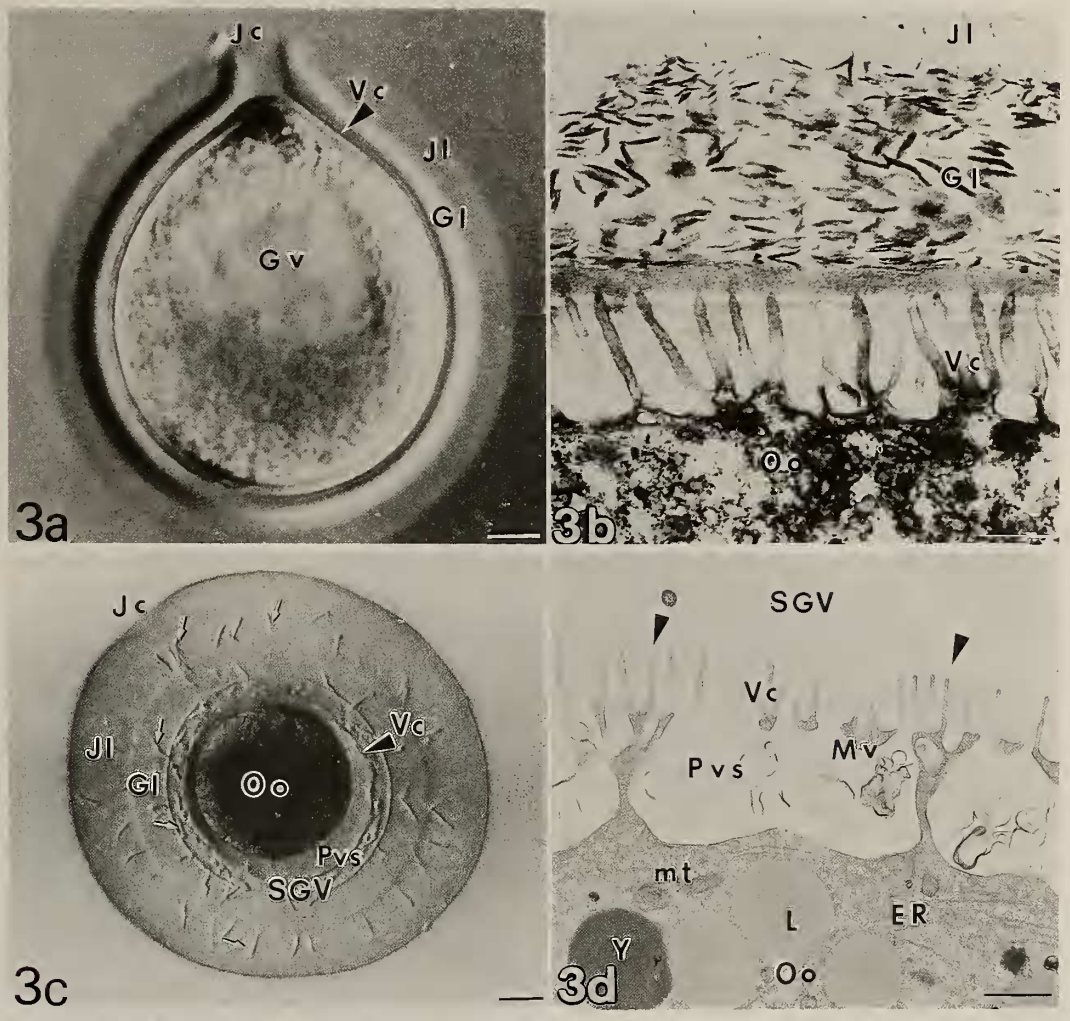


FIG. 3. a: Nomarski micrograph of an oocyte at a meiotic prophase stage of the primary oocyte of *Laternula limicola*. The oocyte is enveloped by the egg investments: jelly layer (Jl), granular layer (Gl) and vitelline coat (arrowhead Vc). The jelly canal (Jc) lacks the jelly and granular layers. Bar: 5 μm.
b: Micrograph (TEM) of the jelly layer (Jl), granular layer (Gl) and vitelline coat (Vc). Bar: 0.5 μm.
c: Nomarski micrograph of an oocyte within 1 min after insemination. The two spaces: SGV and perivitelline space (Pvs), are formed in the egg investments. Many spermatozoa (arrows) entering through the jelly canal and the jell and granular layers into the SGV. Bar: 10 μm.
d: The vitelline coat (Vc) has lifted from the oocyte surface and caused elongation of the oocyte microvilli (Mv). Arrowheads indicate the tips of microvilli remaining in association with the oocyte surface. Bar: 1 μm.
ER: Endoplasmic reticulum, Gv: Germinal vesicle, L: Lipid granule, Oo: Oocyte, mt: Oocyte mitochondria, Y: Yolk granule.

oocytes that are at prophase of meiosis. In longitudinal profile, the primary oocyte is droplet shaped and a jelly canal is visible at the point where the oocyte was attached to the ovarian wall during the process of maturation (Fig. 3a). This jelly canal is similar to that of the sea urchin egg [33]. The primary oocyte is enveloped by multiple layers of egg investments consisting of a jelly layer, a granular layer and a vitelline coat from the outside to the inside. The vitelline coat consists of fine fibrous material associated with the microvilli that are ooplasmic protrusions (Fig. 3b) [37]. When the oocyte is emitted into sea water, the granular layer transforms into a fibrillar structure indistinguishable from the jelly layer in electron micrographs [18]. When all the egg investments swell rapidly due to absorption of sea water, two spaces are newly formed in the egg investments. One space (SGV) develops between the granular layer and the vitelline coat. The other space is the perivitelline space (Pvs) between the vitelline coat and the egg plasma membrane (Figs. 3c, d) [20].

During fertilization, the spermatozoa reach the SGV

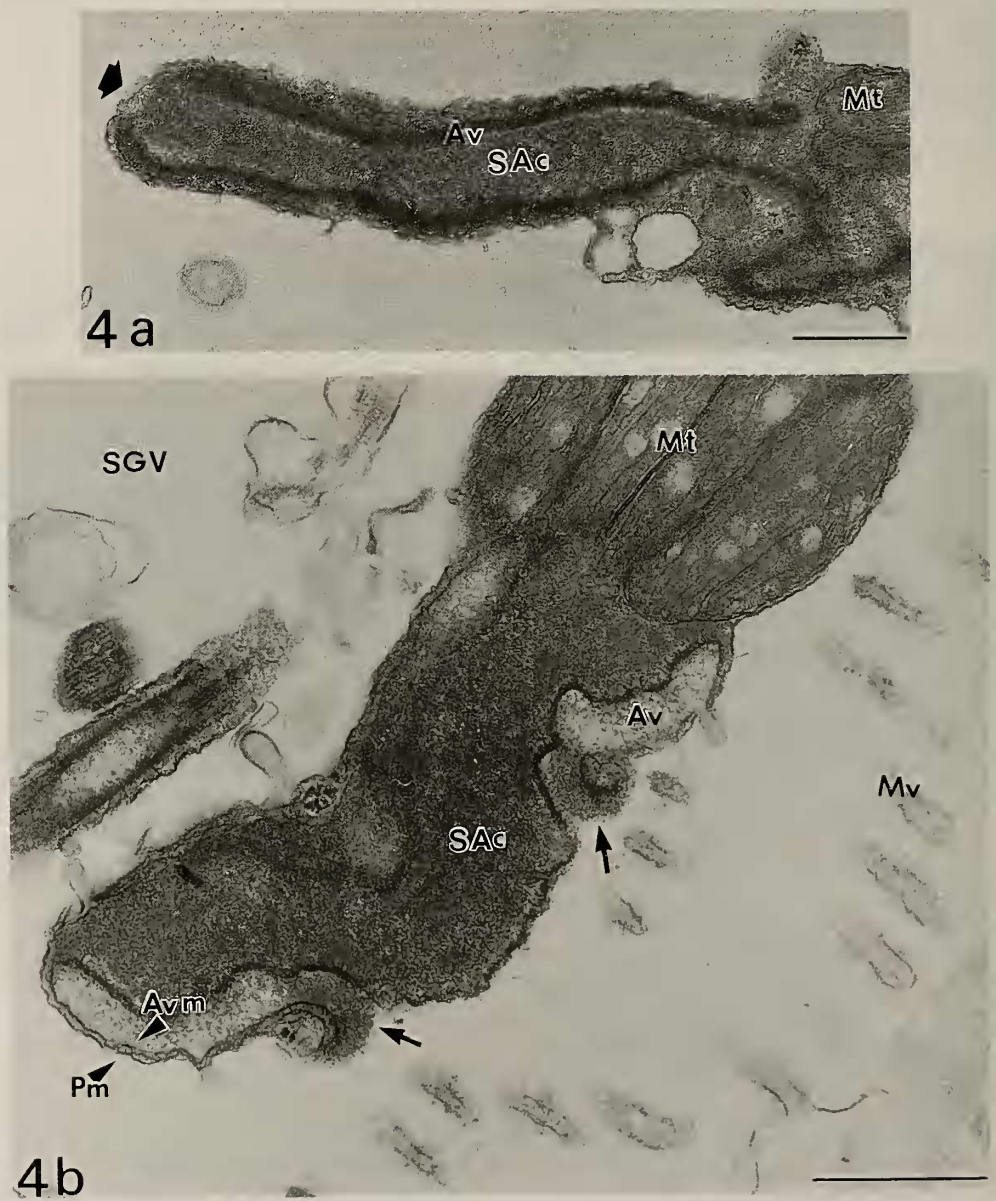
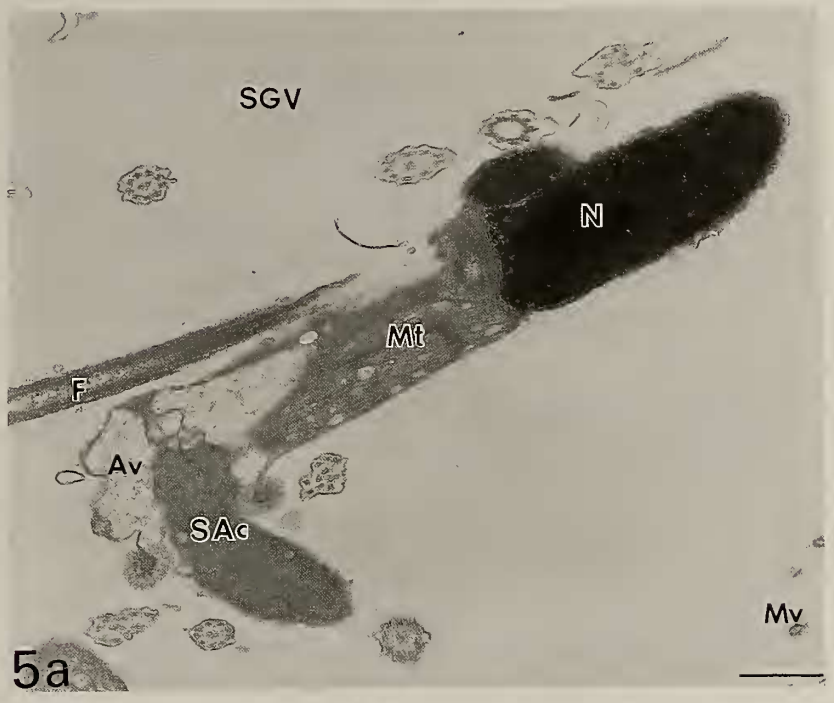


Fig. 4. a: The initial stage of the acrosome reaction. A pit (arrow) is observed at the most posterior point of the acrosomal vesicle (Av). This feature can also be seen in specimens treated with Ionophore A23187. Bar: 0.5 μm.
b: The second stage of the acrosome reaction. The acrosomal vesicle (Av) has swollen. The sperm plasma membrane (arrowhead Pm) and the exterior membrane of the acrosomal vesicle (arrowhead Avm) have fused to each other at the lateral region of the acrosome and opened the acrosome. Some of the acrosomal vesicle material has spread out around the opening. Arrows indicate the extruded materials. The membrane of the subacrosomal region (SAc) has been exposed by this process. Oocyte microvilli (Mv) in diagonal section are visible at the lower right corner of the micrograph. Bar: 0.5 μm.
Mt: Sperm mitochondria, SGV: Space between granular layer and vitelline coat.

sooner by passing through the jelly canal than by way of the jelly layer. The spermatozoa remain in the SGV for a few minutes while beating their tails vigorously. As they pass through the jelly canal, the active movement of the live spermatozoa can be seen clearly with Nomarski optics (Fig. 3c). Many of the spermatozoa in the SGV have a curved head. In electron microscopic observations, the spermatozoa remaining in the SGV appear to have undergone morphological changes in their acrosomes. However, the spermatozoa passing through the jelly and granular layers of the egg investments show no such changes.

2 Acrosome reaction

The morphological changes in the acrosome are divided into the following four stages. In the first stage, a pit forms at the posterior apical part of the acrosomal vesicle (Fig. 4a). In a longitudinal section through this pit, the acrosomal vesicle appears to be divided into two parts. Nearly all the micrographs of spermatozoa treated with Ionophore A23187 also reveal this feature. The entire acrosomal vesicle than swells becoming somewhat rounded and slightly less electron dense than the subacrosomal region. The arrow in Figure 4a shows the discontinuity at the posterior apical tip of the acrosomal vesicle. In the second stage, a morphological change takes place in the ventral lateral part of the acrosomal vesicle. The sperm plasma membrane and the outer membrane of the acrosomal vesicle open and fuse with one another in this ventral lateral region (Fig. 4b). The



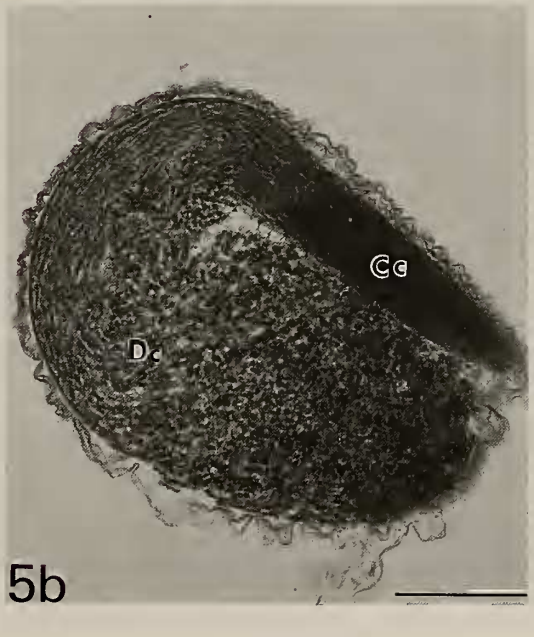


FIG. 5. a: The third stage of the acrosome reaction. A longitudinal section of a reacted spermatozoon in the SGV. The subacrosomal region (SAc) is forced out to the side of the vitelline coat. Note that no extension of an axial rod can be seen in the reacted acrosome. Bar: 0.5 m.

b: Sperm nucleus undergoing the chromatin decondensation. The decondensation of chromatin coincides with the acrosome reaction. The anterior half of nucleus still contains condensed chromatin (Cc). The latter half of the nucleus contains the decondensed chromatin (Dc). Bar: 0.5 μm.

a, b: Note that the sperm head has become round due to folding of the nucleus.

F: Flagellum, Mt: Sperm mitochondria, Mv: Microvilli, N: Nucleus, SGV: Space between granular layer and vitelline coat.

homogeneous electron dense material contained within the acrosomal vesicle exudes from the openings and accumulates at the outer surface of the membrane around the opening (Fig. 4b, arrows). The effusion of materials is accompanied by a regression of the membrane causing the material to spread out along the outer surface. In the third stage, the membrane of the subacrosomal region, the inner acrosomal membrane, is exposed to the SGV. The exposed membrane has the capacity to fuse with the egg plasma membrane. An axial rod cannot be observed as a notable extension from the subacrosomal region, but the subacrosomal region is forced out into the SGV toward the vitelline coat as shown the Figure 5a. In the fourth stage, the exposed and slightly protruding inner acrosomal membrane comes into contact with the tip of one of the intact microvilli that passes through the vitelline coat from the egg plasma membrane. At the point of contact the two membranes rapidly fuse with one another (Fig. 6). A fertilization cone is then formed in the SGV as illustrated in Figures 7a, b. The formation of the fertilization cone is observed within 5 min after insemination.

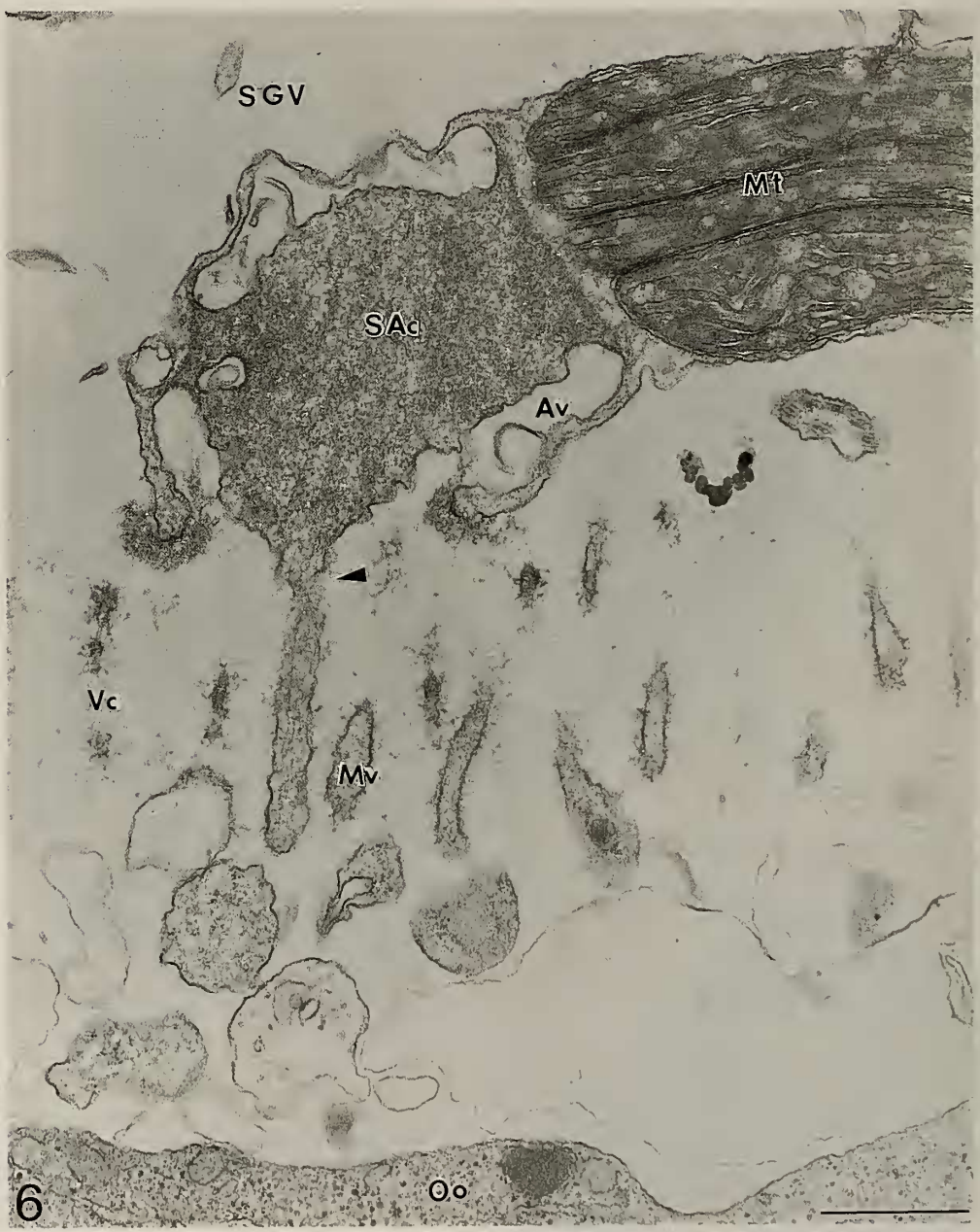


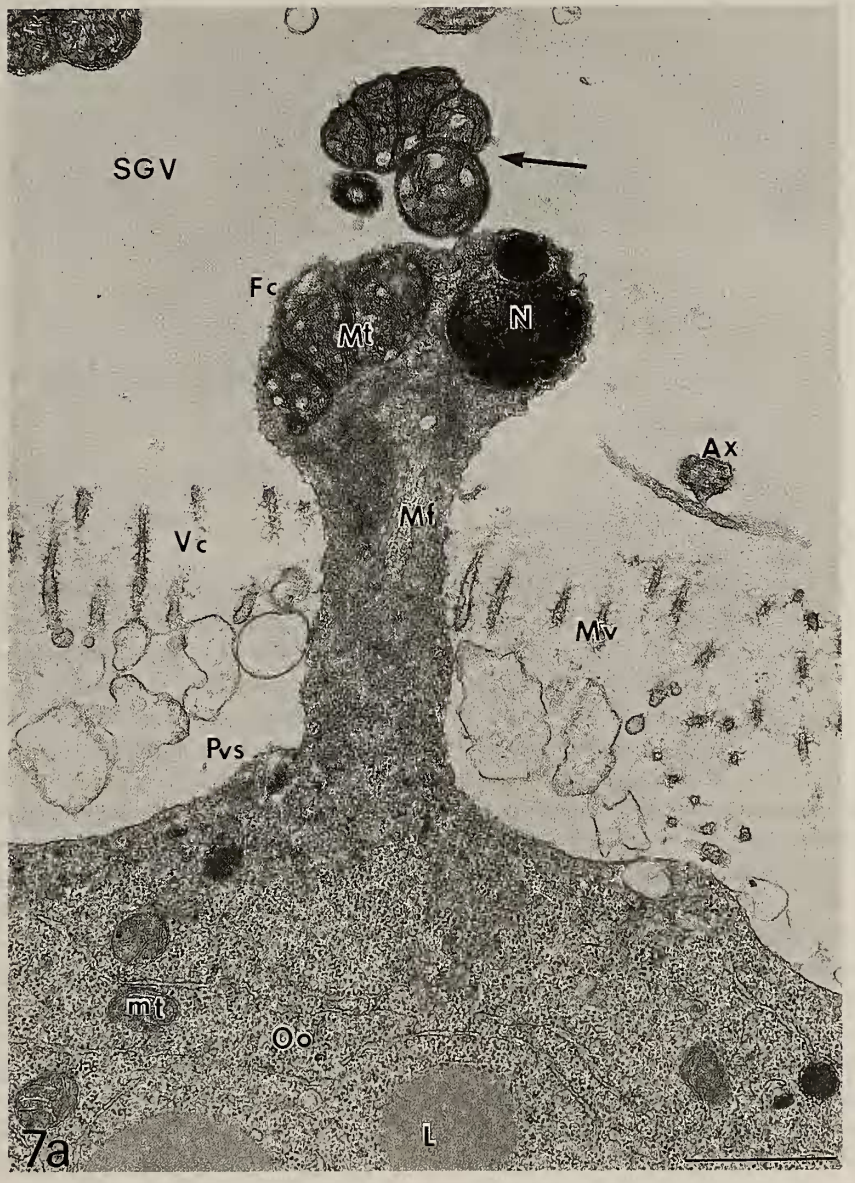
Fig. 6. The final stage of the acrosome reaction. The subacrosomal membrane of the spermatozoon has contacted a microvillus passing through the vitelline coat (Vc). This spermatozoon cannot immediately penetrate into the oocyte. But, if the microvillus which contacts the reacted subacrosomal region (SAc) is in continuous contact with the egg plasma membrane, the spermatozoon can probably be incorporated into the oocyte. An arrowhead indicates the point of membrane fusion between the microvillus and subacrosomal membranes. Bar: 0.5 μm.
Av: Acrosome vesicle, Mt: Sperm mitochondria, Mv: Microvilli, Oo: Oocyte, SGV: Space between granular layer and vitelline coat.

At the same time, the sperm head undergoes unique structural change also. The nucleus folds in half toward the dorsal side of the head, and then the sperm head become round (Fig. 5a, b). The condensed sperm chromatin begins to decondense beginning at the basal region of the nucleus bordering the mid-piece mitochondria. The zone of decondensed chromatin expands gradually until nearly one half of the nucleus is decondensed (Fig. 5b). The chromatin decondensation occurs at the same time as the acrosome reaction.

3 Sperm penetration

It is very difficult to find the fertilizing spermatozoon in electron microscopic thin sections. The steps in the process of sperm penetration are shown in Figures 7a, b and Figure 8. In *L. limicola,* sperm incorporation begins at the posterior region of the spermatozoon. The sperm organelles are incorporated into the ooplasm in the following order: the microfilaments of the subacrosomal region are incorporated first and are immediately followed by the five sperm mitochondria, the pair of centrioles and the axial filament. The sperm nucleus with partially decondensed chromatin is the last to enter the ooplasm closely following the other organelles (Fig. 8). After penetration, the nucleus moves away from the mitochondria at the base of the fertilization cone, migrates into the ooplasm and developes into the sperm pronucleus.

Within a few seconds after insemination, before the SGV and Pvs have completely formed in the egg investments, the spermatozoa which have reached the oocyte surface by directly passing through the egg investments or by way of the jelly canal enter the ooplasm polyspermically beginning at their heads. This form of sperm entry bears a resemblance to phagocytosis. The acrosomes of these spermatozoa remain unchanged. Since these spermatozoa cannot fuse with the egg plasma membrane due to their abnormal entry, they are ejected from the oocyte surface into the SGV as rapid



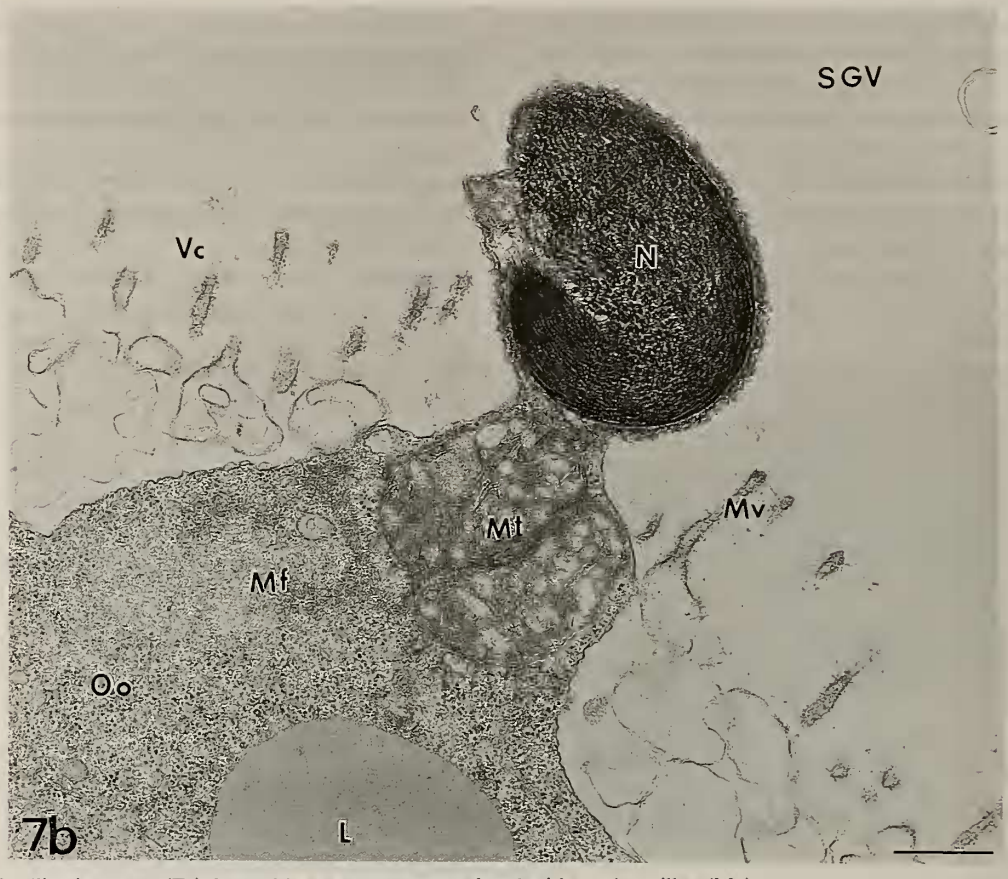


Fig. 7. Fertilization cone (Fc) formed by a spermatozoon fused with a microvillus (Mv).
a: The fertilization cone has formed through and beyond the vitelline coat (Vc). The subacrosomal microfilaments (Mf) precede the mitochondria and nucleus in the fertilization cone. An arrow indicates the mitochondria (Mt) of another spermatozoon. Bar: 1 μm.
b: A spermatozoon of which the mitochondrial mid-piece has traversed the vitelline coat (Vc). The nucleus (N) follows behind the mitochondria (Mt), but it is still outside the vitelline coat. Note the disposition of the microfilaments (Mf), mitochondria (Mt) and nucleus in the fertilization cone. The fertilization cone is gradually shrinking in extent until at last the nucleus enters into the oocyte (Oo). Bar: 0.5 μm.
Ax: Axial filament, L: Lipid granule, mt: Oocyte mitochondria, Pvs: Perivitelline space, SGV: Space between granular layer and vitelline coat.

changes in the egg investments occur.

The main findings of this study are outlined diagrammatically in Figure 9.

DISCUSSION

In this study, the processes of acrosome reaction, the contact and fusion of the sperm plasma membrane with the egg plasma membrane, and sperm penetration into the oocyte were observed at the ultrastructural level in the bivalve *Laternula limicola*.

According to Kubo [25], the mature spermatozoon does not have a typical acrosome situated anterior to the nucleus. However, in the spermatid, a structure similar to an acrosome and derived from the Golgi complex is temporarily located anterior to the nucleus. In late spermiogenesis, this acrosome-like structure finally moves to the posterior region of the mitochondrial mid-piece along the lateral side of the nucleus. Its formation is almost identical to that of a typical acrosome. Due to this structural shift to the posterior mid-piece, Kubo assigned it the new name of "temporary acrosome" and then suggested that the temporary acrosome is a kind of degressive acrosome.

Our observations of the ultrastructural features of mature spermatozoa are nearly consistent with Kubo's description [25]. The authors found that during fertilization the temporary acrosome changed in structure. Moreover, our electron microscopic observations revealed that sperm penetration occurs through this temporary acrosome. The morphological changes in this acrosome correspond closely to the general concept of the acrosome reaction as described by Dan [9, 10] and Colwin and Colwin [7, 8], but the structure of the reacted acrosome lacks the extension of an axial rod as seen in other invertebrate species such as the mussel [36], the sea urchin [12], the asteroid [47] and the abalone [46, 54]. The microfilaments that appear in the fertilization cone may be reorganized from invisible fibrillar elements included in the subacrosomal region. These are presumably similar to

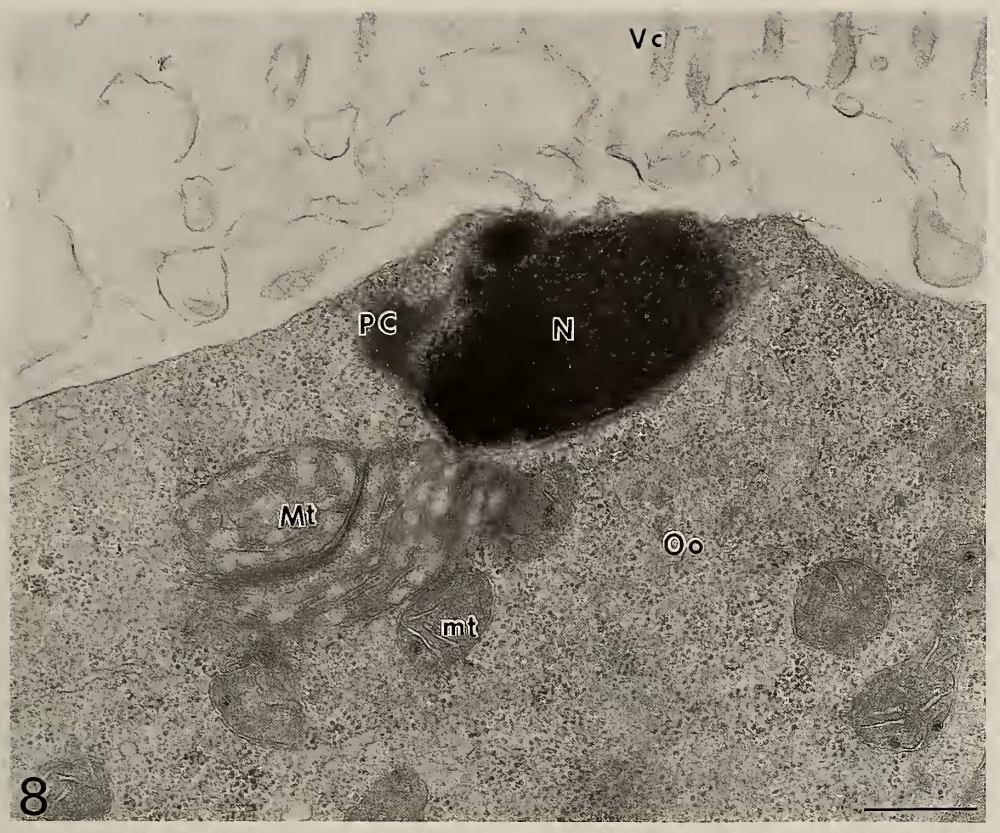


FIG. 8. The incorporated spermatozoon. The sperm mitochondria (Mt) are larger than the oocyte mitochondria (mt). Bar: 0.5 μm.
N: Nucleus, Oo: Oocyte, Pc: Proximal centriole, Vc: Vitelline coat.

the acrosomal actin filament of the axial rod extending from the reacted acrosome as reported previously by Tilney [51, 52] and Shiroya [46].

Further observation is necessary to elucidate the triggers [11] for this acrosome reaction and to obtain detailed information on the significance of the pit formed in the first stage of the acrosome reaction.

It is a matter of common knowledge that the spermatozoa of a number of invertebrate species enter the egg or the oocyte from the apex of the nucleus [1, 6, 14, 15, 35, 44, 45, 50] or in mammals from the lateral side of the nucleus [56]. This pattern has been observed in almost all studies on fertilization including those of species in which the spermatozoa lack an acrosome [4, 21, 23, 28, 40].

The mature spermatozoon of *L. limicola* not only possesses an acrosome at the posterior end of the mid-piece, in contrast to its ordinary position, but also fuses with the egg plasma membrane at the ventral lateral region of the reacted acrosome during fertilization of mature egg. The spermatozoon enters the oocyte through a fertilization cone formed by fusion betwkeen the inner acrosomal membrane and the plasma membrane of an oocyte microvillus. The sperm organelles enter the oocyte in reverse order to that observed in other species; that is, the nucleus follows the mid-piece mitochondria. This mode of sperm penetration has not been previously reported. This is the first report of this unusual mode of fertilization. The fertilized eggs used in this study continued to develop through the D-shaped veligier larvae phase as previously reported by the authors [19]. Thus, participation of the posterior acrosome in fertilization is normal for this species. The authors suggest that the acrosome of *L. limicola* spermatozoa ought to be referred to as a "typical acrosome" rather than the term "temporary acrosome" coined by Kubo [25].

In 1979, Kubo *et al.* have reported their ultrastrutural studies on sperm-egg interaction at the time of fertilization from a viewpoint that the mature spermatozoon in *L. limicola* has an atypical acrosome [27]. Their observations were as follows. The temporary acrosome does not change morohollogically. The sperm penetrates into the egg leaving the mid-piece with its mitochondria and temporary acrosome on the outside of the egg. The sperm head is engulfed by the egg surface, but the sperm plasma membrane and egg plasma membrane do not fuse. Thus, the temporary acrosome has no function in sperm-egg interaction.

In the present studies, the authors found that the posterior acrosome undergoes the acrosome reaction and enters the egg along with the mitochondria, nucleus and tail. Our observations of the fertilization process in *L. limicola* did not coincide with the description presented by Kubo *et al.* [27].

The role of the posterior acrosome in the process of fertilization is one of the more intriguing questions regarding

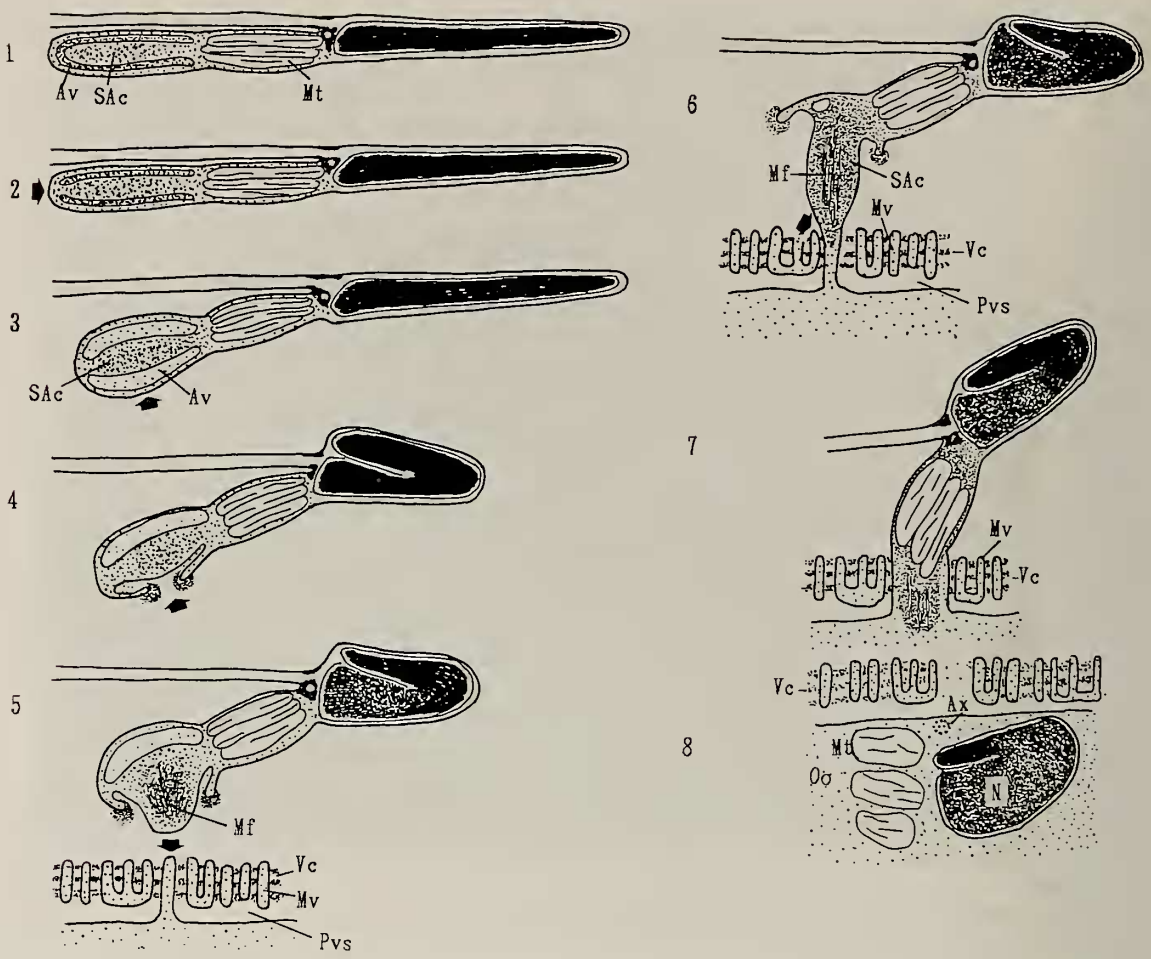


FIG. 9. Diagrammatic illustration of the spermatozoon and successive steps of sperm pentration into the oocyte.
1: Intact spermatozoon.
2: A spermatozoon at the beginning of the acrosome reaction. An arrow indicates the pit formed at the posterior apical part of the acrosoma vesicle.
3: An arrow indicates the ventral lateral part of the acrosome.
4: An arrow indicates the opening at the lateral part of the acrosome and the acrosomal vesicle contents that have been extruded from the opening.
5: The spermatozoon approaching the vitelline coat. An arrow indicates the point where the subacrosomal region will be able to contact the microvilli.
6: An arrow indicates the membrane fusion between the subacrosomal region and the tip of a microvillus in continuous contact with the egg plasma membrane.
7: The formation of the fertilization cone.
8: The incorporated spermatozoon.
Av: Acrosomal vesicle, Ax: Axial filament, Mf: Microfilament, Mt: Sperm mitochondria, Mv: Miocrovilli, N: Nucleus, Oo: Oocyte, Pvs: Perivitelline space, SAc: Subacrosomal region, Vc: Vitelline coat.

the mode of sperm penetration, but as yet relatively little is known about acrosomes situated at the posterior region of the sperm mid-piece as reported by Kubo in the bivalve, *Laternula limicola* [25], Kubo and Ishikawa in the bivalve, *Lyonsia ventricosa* [26], Reger in the tick *Amblyomma dissimili* [41] and Bawa in the fire-brat insect, *Thermobla domestica* [2]. The significance of the posterior relocation of the acrosome during late spermiogenesis with regard to sperm penetration has not been defined for other species. Further studies in species with spermatozoa similar to those of *L. limicola* would be necessary for consideration of any phylogenetic comparative significance.

One notable observation is that the sperm nucleus folds in half dorsaly and begins decondensation of its chromatin while the acrosome reaction is progressing. According to Longo [29] and Longo and Anderson [30], the condensed chromatin generally undergoes decondensation after the sperm nucleus has been incorporated into the ooplasm. However, this phenomenon in *L. limicola* is different from the ordinary form of chromatin decondensation. In this study, it was not possible to determine why the nucleus folded in half and why the condensed chromatin started to decondense before sperm incorporation. It may be interesting to determine whether or not the acrosome reaction is directly

related to chromatin decondensation, although these two events are quite distinctive from each other.

Schatten and Mazia [45] have described a phenomenon in fertilization of the sea urchin egg in which the incorporated spermatozoon rotates to position its centriole end to face inward. In the case of the *L. limicola* spermatozoon, it does not seem necessary to rotate 180°, because the sperm enters with the mid-piece facing the oocyte. A full-process of male pronuclear development has not described in this study.

The SGV, which is newly created in the egg investments just before fertilization, provides the place where the acrosome undergoes morphological change. The reacted acrosome contacts and fuses with the egg plasma membrane and the fertilization cone forms in the SGV. It appears that the oocyte must secrete into the SGV some materials which induce the sperm to activate. The creation of the Pvs in the egg investments has a restraining effect on polyspermic penetration, since the Pvs interrupts the connections between most microvilli and the egg plasma membrane. In consequence, the success of fertilization depends on whether or not the reacted acrosomal region touches a microvillus which is still in continuous contact with the egg plasma membrane.

This report has not made mention of the role of the material contained in the acrosomal vesicle. However, there is no doubt that it participates in the fertilization of this species as a sperm agent (lysin? [10], bindin? [55]) acting on the oocyte. The spermatozoon does not need to disperse this material within the jelly and granular layers of the egg investments, because the spermatozoa can pass through these layers without undergoing the acrosome reaction. The authors tried to immunocytochemically test using an antibody (provided by Haino-Fukushima [16]) against the 15.5K vitelline coat lysin extracted by Haino-Fukushima from spermatozoa of the abalone, *Haliotis discus,* to determine whether it would cross-react immunocytochemically with any antigen in a thin section of a *L. limicola* acrosomal vesicle. The protein A-Gold technique was used [16]. The gold label was not found in the region of the *L. limicola* acrosomal vesicle. The chemical characterization and role of the acrosomal vesicle contents in *L. limicola* spermatozoa are subjects of intense and continuing interest.

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